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Original Research Article

Comparative Adsorption of Spiramycin on Veegum®, Activated Charcoal and *Garcinia kola* Heckel (Guttiferea) Seed

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Abstract

Purpose: *To investigate the adsorptive interaction of Garcinia kola with spiramycin, since the kola is widely chewed as a tonic and spiramycin attains high concentrations in saliva.*

Methods: *Spiramycin solutions of different concentration were added to a fixed mass of Garcinia kola (200 mg), activated charcoal or Veegum®. Shaking was carried out at room temperature after which the dispersion was filtered and the filtrate assayed for residual drug concentration. The process was repeated under different equilibrium conditions of pH and ionic strength. The adsorption data obtained for the three adsorbents were analyzed using Langmuir and Freundlich's plots.*

Results: *At neutral pH, drug adsorprtion by Garcinia kola, activated charcoal and Veegum® were 67, 54 and 71 %, respectively; differences in adsorption was not significant (p = 0.09). However, the other two adsorbents exhibited adverse adsorption characteristics in terms of negative adsorption capacity (-5.78 mol.kg-1) and constant (-1141 mol-1L). For each of the adsorbents, pH and ionic strength affected the extent of adsorption, due to their effect on adsorbent surface charge. Correlation with Langmuir and Freundlich relationships were poor, the correlation coefficient for the latter being 0.97, 0.894 and 0.351 for Garcinia kola, Veegum® and activated charcoal, respectively.*

Conclusion: *The study reveals that Garcinia kola significantly adsorbs spiramycin under alkaline conditions comparable to salivary pH, and therefore should not be taken concurrently with the drug in order to minimize reduction in drug levels.*

Keywords: *Garcinia kola, Spiramycin, Adsorption, Antidote, Interaction, Langmuir plot, Freundlich's plot*

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INTRODUCTION

Activated charcoal and Veegum® are used extensively as adsorbents in different pharmaceutical and chemical applications. Activated charcoal, due to its high adsorptive capacity, has long found use in the removal of colours from solutions [1,2], pyrogens from injections [3,4] and in the management of acute poisoning [5-7]. Though activated charcoal is so

popular, clays are also known to have high adsorptive capacities due to their colloidal dimensions which result in high exposed surface areas; they also have high ion exchange capacities as a result of negative charges in solution. Veegum® (aluminium magnesium silicate) occurs either as small white flakes or micronized powder, which swells in water forming colloidal dispersions.

Garcinia kola, a flowering tree (family *Clusiacae*) also known as "bitter or false kola" is an economic tree growing in humid forestlands of West and Central Africa with a long history of use in traditional medicine [8]. The seeds are about 4 - 5 cm long, elliptically shaped and covered with a brown testa. The seeds are harvested from decayed fruits and are widely chewed as a tonic, as part of the social cultures of many traditional societies. Most common medical uses are as remedy for cough and mouth infections [8], antidote for poisons [9] and anti-hepatotoxic [10]. The use of the seed in mouth infections and cough conditions holds significant prospects of an interaction with drugs found in high concentration in saliva, such as spiramycin. The latter, derived from *Streptomyces ambofaciens*, is orally absorbed and attains salivary concentrations about 1.3 to 4.8 times higher than in serum [11], and so holds significant potentials for interaction with the *Garcinia kola*. In this study, the adsorption of spiramycin by the two common agents being activated charcoal and Veegum® are compared to that of *Garcinia kola*. Significant interaction may lead to reduced salivary levels of the drug and help in initiation and development of drug resistance.

EXPERIMENTAL

Materials

Rovamycin® (Rhone-Poulenc), sodium chloride (Merck), aluminium magnesium silicate (Veegum®) and activated charcoal (Norit Co., USA) were procured from their suppliers. Dried *Garcinia kola* seeds were procured from a local market in Nsukka, Enugu State, Nigeria, in September, 1999. The material was authenticated by Mr. Alfred Ozioko, a taxonomist with the International Centre for Ethnomedicine and Drug Development, Nsukka, Enugu State. Reference samples have are stored in the herbarium of the Department of Pharmacognosy, University of Nigeria, Nsukka, with voucher no UNN/PCOG/072. Distilled water was obtained from an all-glass still in our laboratory. All other materials were used as sourced from their suppliers without any modification.

Preparation of test adsorbent

The brown testa was removed from the seeds, which were then dried in an oven (Unitemp B &T Searce Co.) at 40 ^{o}C for 2 days. The dry nuts were pulverized in an end runner mill and then sieved. The pulverized mass was then heated in an oven at 120 ^{o}C for 5 h. Both activated charcoal and Veegum® were similarly heated before use.

Extraction of spiramycin (Rovamycin®)

Ten tablets of Rovamycin® were crushed in a clear dry porcelain mortar and the powdered mass was extracted with methanol and the filtrate filtered through a Whatman no. 1 filter. Gentle heating was then applied to recover the crystalline spiramycin.

Determination of effect of initial concentration of adsorbate

All the adsorbents were first heated in a hot air oven at 120 ^{o}C for 5 h, after which they were allowed to cool down. Varying concentrations of spiramycin ranged between 50 mg% and 200 mg% were prepared in 0.1 N HCl. Garcinia kola (200 mg) was weighed out into five 25 ml volumetric flasks and 20 ml of spiramycin solution (one for each concentration) was introduced. The fifth flask contained no drug. The flasks were stoppered and shaken for three hours at room temperature in a GallenKamp shaker (England). The procedure was repeated for Veegum® and activated charcoal. At the end of the period, the amount of drug remaining in solution was determined by reading the absorbance of the filtrate in a spectrophotometer (sp8-100) at 230 nm and then calculating for the equivalent concentration using a calibration plot obtained in 0.1 N HCl.

Determination of effect of pH on adsorption

Different 50 mg% spiramycin solutions were prepared in buffer of pH values of 1.2, 7.0 and 10.0. *Garcinia kola* (200 mg) was placed in a 25 ml volumetric flask followed by addition of 20 ml of the solution. A control system containing an equal quantity of buffer and adsorbent was also prepared for each of the three buffers. The flasks were then stoppered and shaken for 3 h at room temperature as before. At the end of the period, the dispersions were filtered and analyzed for content of spiramycin.

Determination of effect of ionic strength

The spiramycin solution (50 mg%) was prepared in the presence of varying concentrations of sodium chloride, namely 0.1 N, 0.5 N, and 5.0 N. The volume of drug solution and amount of adsorbent were the same as in the previous tests. After allowing for 3 hour equilibration with shaking, the equilibrium drug concentration was determined as before.

Effect of adsorption on surface tension

The surface tension was measured for four concentrations of spiramycin (50, 100, 150 and 200 mg %) before shaking with each adsorbent system and also after shaking and filtering, in a Cenco-Du Nouy interfacial tensiometer at room temperature. A mean of three readings was obtained.

Statistical analysis

Statistical analysis was carried out using SPSS version 16.0. Statistical level of significance was tested using Friedman two-way ANOVA with $p < 0.05$ considered significant.

RESULTS

Of the three adsorbents tested, the adsorptive capacities for spiramycin are in the order: Veegum® > activated charcoal > *Garcinia kola*. Langmuir plots (not shown) for the equilibrium adsorption conditions with the different adsorbents generally had poor linear correlations. Freundlich plot for *Garcinia kola* is presented in Figure 1 while the adsorption constants for all the isotherms are presented in Table 1. The surface concentration increased with initial solute concentration for the three adsorbents (Table 2). Both pH and ionic strength affected the extent of adsorption for the three adsorbents. From the Friedman two-way ANOVA by ranks, there were no statistical differences between the percentage adsorption for the three adsorbents (p = 0.097). The extents of adsorption also did not vary significantly between the three with ionic strength (p = 0.717). Only activated charcoal produced a surface tension lowering effect; surface tension increased with surface concentration with the other adsorbents.

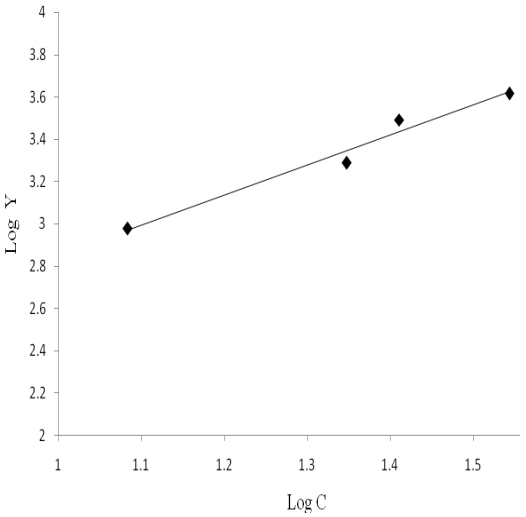

Figure 1: Freundlich plot for adsorption of spiramycin on *Garcinia kola*. Mass of *Garcinia kola*: 0.2 g; volume of spiramycin solution: 25 ml. R^2 equals 0.97, Y in $mol.kg^{-1}$, C in $mol.L^{-1}$

DISCUSSION

Adsorption isotherms

To better understand the relationship between the adsorbents and spiramycin, use was made of the Langmuir equation (Equation 1), which relates the equilibrium concentration (C in $mol.L^{-1}$) of drug, the amount of drug adsorbed per unit mass of adsorbate (Y in $mol.kg^{-1}$) and the adsorptive capacity of the adsorbate (Y_m in $mol.kg^{-1}$) was employed.

$$C/Y = 1/KY_m + C/Y_m \quad (1)$$

K (unit of $(mol^{-1}.L)$ is a constant which is unique for the spiramycin-adsorbent system at the test temperature.

Table 1: Adsorption constants for spiramycin on three different adsorbents

Adsorbent	K ($mol^{-1}L$)	Y_m ($mol.kg^{-1}$)	R^2 (Langmuir)	R^2 (Freundlich)	n (K)
Activated charcoal	28, 169	3.86	0.574	0.351	1.94 (738)
Veegum®	80, 000	6.94	0.700	0.894	2.35 (2685)
Garcinia kola	-1141	-5.78	0.764	0.97	0.702 (26.755)

Table 2: Adsorption of spiramycin as a function of starting parameters

Variable	Bound spiramycin (%)		
	Activated charcoal	Veegum®	*Garcinia kola*
Initial conc. (mg %)			
50	91.3	99.7	75.8
100	78.7	98.4	77.7
150	87.7	98.9	82.8
200	94.9	98.5	82.5
pH			
1.2	88.2	94.5	69.3
7.0	54.2	83.4	71.8
10.0	76.5	91.6	67.4
Ionic strength (N)			
0.1	94.2	93.8	80.3
0.5	94.4	97.9	92.9
1.0	94.6	97.2	97.5

Freundlich equation was also utilized in the analysis.

$$\text{Log } Y = \log K + 1/n \log C \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

where K and n are constants unique for the spiramycin-adsorbate system at the test temperature (25 ^{o}C).

Langmuir isotherms generally had poor linear regressions, as evident in the correlation coefficients obtained. The reasons are not fully understood. However, the inapplicability of isotherm models, particularly the Langmuir isotherm to several solution phase adsorption data is well known and had been at the centre of attempts to modify the equation to better fit solution phase data [12]. In fact, poor linear correlation of between 0.37 and 0.85 comparable to ours was reported in the application of the Langmuir equations to solution phase adsorption of orthophosphate on titanium [13]. These difficulties in applying the Langmuir equations have been attributed to heterogeneity of binding sites and to charge balancing effects occurring in solution - adsorption of one species in solution may lead to displacement of another previously adsorbed species in order to create charge balance, which violates the independence of individual processes assumed in the Langmuir postulates [12,14]. This deviation increases with concentration of adsorbate. The heterogeneity of surfaces encountered with many solid adsorbents has also been advanced as a reason, as it means that that equality of binding sites, a fundamental assumption of Langmuir, no longer holds. In consequence, we have reported in Table 1 parallel fits obtained from Freundlich's plot, which is believed to generally better fit with solution phase data [12]. Freundlich plot for *Garcinia kola*, which has a better regressional fit, is presented in Figure 1. While this holds true for *Garcinia kola* and Veegum®, it is not the case with activated charcoal, where the lowest correlation coefficient of 0.351 is seen. The negative adsorption capacity of -5.78 mol.Kg^{-1} and also the negative equilibrium constant obtained with *Garcinia kola* imply that the process is not thermodynamically favourable [15] at the test temperature.

The solute removal efficiency increased with increase in the amount of spiramycin. This was quite unexpected because even though the rate and extent of adsorption is known to increase with solute concentration, the fraction of adsorbate removed by a particular mass of adsorbent is known to decrease with increasing solute concentration [16-18] and so adsorption is more efficient for dilute solutions. The explanation offered here is that the increase in the solute removal efficiency with initial concentration of solute for the three adsorbents is only possible because the initial concentrations tested are rather low in relation to the adsorption capacities of each of the adsorbents at the test temperature. Indeed, the surface concentration obtained for 200 mg% is more than 1500 times lower than the Y_m for activated charcoal. At low concentrations, the number of molecules of adsorbate is much lower in comparison to the number of active sites available for binding, leading to higher removal efficiency. At higher initial adsorbate concentrations, this advantage is lost.

Extremes of pH favour adsorption for activated charcoal and Veegum®. The pH effects may be understood in terms of solubility and charge relationships. Spiramycin has two tertiary amine centres and a pKa of 8.0. Low pH promotes solubility and may cause protonation. For

uncharged adsorbents, the uncharged forms of adsorbates are more readily adsorbed. In the case of activated charcoal, the reduced adsorption around neutral pH may point to a charge repulsion effect near the point of zero charge (pH_{pzc}) of the activated carbon where the adsorbate acquires a net positive charge. For spiramycin on a negatively charged surface like Veegum®, electrostatic attraction between adsorbate and negatively charged adsorbent may lead to improved adsorption, which is also the case. At high pH values, the explanation offered is that the fairly high adsorption relative to neutral pH is due to reduced solubility. Thus, the adsorption at high pH is a solubility effect, while that at low pH is a charge effect. The highest removal efficiency of nearly 100 % obtained at low pH for Veegum® points strongly supports a charge effect, in which the clay is negatively charged in solution, opposite to the charge on the drug.

In comparing our results with those of others, is difficult to draw a general rule for the effect of pH on adsorption. For acidic dyes, pH increase has been reported to both increase the removal efficiency [18], and to decrease it [19].

The influence of ionic strength is most pronounced with *Garcinia kola*, where higher removal efficiency is seen with increasing ionic strength. The explanation suggested is that a high concentration of sodium ions can be expected to influence both the ionization of other co-solutes as well as their solubility. The latter principle hold true for macromolecules and is frequently employed in "salting out" processes. Ionic strength significantly neutralizes any repulsive charge effects and can be expected to enhance adsorption, depending on whether the adsorbent is charged. Though high ionic strength is generally believed to favour adsorption [19], this relationship is complex because it is always interlinked with pH and charge effects, and both decreasing and increasing adsorption can occur with changes in ionic strength, depending on pH and other influences [20]. For a charged adsorbent where ion exchange influences are pronounced, the high concentration of sodium ions might be expected to compete with any protonated forms of spiramycin for the electrical double layers and ionic adsorption sites; however, indeed, the protonation of spiramycin in conditions of high sodium ion activity is very low, so that this competition may not exist. Therefore, the unprotonated form is removed by non-electrostatic mechanisms, being why the removal efficiency did not fall significantly with increase in ionic strength for Veegum®. The same influences seen with pH and ionic strength were reported by Bjelopavlic *et al* [20], who investigated the adsorption of reactive dyes on activated carbon.

With activated charcoal, surface tension decreased with the surface concentration of spiramycin. Hydrophobic adsorption is driven by interfacial tension, and so this phenomenon is quite expected. For Veegum® where surface tension increased with surface concentration of spiramycin, the inference drawn is that the role of interfacial tension may be less pronounced in adsorption, and adsorption may have been mediated by electrostatic effects, as previously discussed.

CONCLUSION

The study reveals that *Garcinia kola* adsorbs spiramycin and therefore concurrent use or administration of the two preparations should be avoided. However, it would be better to counter spiramycin poisoning or overdosage with Veegum or activated charcoal, due to their larger adsorptive capacities. The extent of effect of concurrent use of *Garcinia kola* on the pharmacokinetics of spiramycin *in vivo* needs to be evaluated.

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